In re Application of : Scott W. Van Arsdell, *et al.*
U.S. Serial No.: 08/914,332
For: **OVERCOMING DAPA AMINOTRANSFERASE BOTTLENECKS IN BIOTIN VITAMERS BIOSYNTHESIS**

PROPOSED CLAIMS

****FOR DISCUSSION ONLY - NOT FOR ENTRY ON THE RECORD****

Claim 1. (**Currently amended**) A method of producing a biotin vitamer by:

(a) culturing a bacterium comprising a ***Bacillus*** lysine-utilizing diaminopelargonic acid (DAPA) aminotransferase, said culturing taking place in an environment wherein lysine, a lysine analog, or a lysine precursor is exogenously added to the culture to provide a concentration of at least 10 mmoles lysine, lysine analog, or lysine precursor per liter of culture during the entire culturing step; and

(b) recovering said biotin vitamer.

Claim 2. (**Currently amended**) A method of producing a biotin vitamer by:

(a) culturing a bacterium comprising a ***Bacillus*** lysine-utilizing DAPA aminotransferase, wherein the lysine biosynthetic pathway is deregulated in said bacterium; and

(b) recovering said biotin vitamer.

Claim 3. **(Currently amended)** The method of claim 1 in which the bacterium is engineered to overproduce a *Bacillus* lysine-utilizing DAPA aminotransferase.

Claim 4. **(Currently amended)** The method of claim 2 in which the bacterium is engineered to overproduce a *Bacillus* lysine-utilizing DAPA aminotransferase.

Claim 5. **(Original)** The method of claim 2 or claim 4, wherein lysine, a lysine analog, or a lysine precursor is exogenously added to the culture.

Claim 6. **(Previously amended)** The method of claim 2 or claim 4, in which lysine, a lysine analog, or a lysine precursor is exogenously added to the culture to provide a concentration of at least 10 mmoles lysine, lysine analog, or lysine precursor per liter of culture during the entire culturing step.

Claim 7. **(Original)** The method of claim 1, claim 2, claims 3, or claim 4, in which the biotin vitamer is biotin, dethiobiotin, or diaminopelargonic acid (DAPA).

Claim 8. **(Original)** The method of claim 1, claims 2, claim 3, or claim 4, in which the biotin vitamer is dethiobiotin, and, after recovering the dethiobiotin,

the method further comprises converting the recovered dethiobiotin to biotin by a separate fermentation, biochemical reaction, or chemical reaction, and recovering biotin.

Claim 9. **(Original)** The method of claim 1, claim 2, claim 3, or claim 4, in which the bacterium is resistant to a lysine analog.

Claim 10. **(Original)** The method of claim 9, wherein said analog is S-2-aminoethyl-L-cysteine (AEC).

Claim 11. **(Previously amended)** The method of claim 1 or claim 2, wherein at least one biotin synthetic pathway step, in addition to expression of a gene encoding a DAPA aminotransferase, ~~the *bioA* gene~~ is deregulated in said bacterium.

Claim 12. **(Original)** The method of claim 1, claim 2, claim 3, or claim 4, in which the biotin vitamer is biotin, and the method comprises recovering and purifying the biotin.

Claim 13. **(Previously amended)** The method of claim 1, claim 2, claim 3, or claim 4, wherein said bacterium is further engineered to produce a S-adenosylmethionine (SAM)-utilizing DAPA aminotransferase.

Claim 14. **(Original)** The method of claim 13 in which methionine, S-adenosylmethionine (SAM), or an analog of SAM is added to the culture.

Claim 15. **(Original)** The method of claim 13 wherein lysine, a lysine analog, or a lysine precursor is added to the culture.

Claim 16. **(Original)** The method of claim 14, wherein lysine, a lysine analog, or a lysine precursor is added to the culture.

Claim 17. **(Previously amended)** The method of claim 15 in which lysine or a lysine analog exogenously added to the culture provides a concentration of at least 10 mmoles lysine or lysine analog per liter of culture during the entire culturing step.

Claim 18. **(Previously amended)** The method of claim 16 in which lysine or a lysine analog exogenously added to the culture provides a concentration of at least 10 mmoles lysine or lysine analog per liter of culture during the entire culturing step.

Claim 19. **(Original)** The method of claim 13 in which the biotin vitamer is biotin, dethiobiotin, or diaminopelargonic acid (DAPA).

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Claim 20. **(Original)** The method of claim 13 in which the biotin vitamer is dethiobiotin, and, after recovering the dethiobiotin, the method further comprises converting the recovered dethiobiotin to biotin by a separate fermentation, biochemical reaction, or chemical reaction, and recovering biotin.

Claim 21. **(Currently amended)** The method of claim 13 wherein at least one biotin synthetic pathway step, other than expression of a gene encoding a DAPA aminotransferase, ~~the *bioA* gene~~ is deregulated in said bacterium.

Claim 22. **(Original)** The method of claim 13 in which the biotin vitamer is biotin, and the method comprises recovering and purifying the biotin.